Exhibit 4

Highly Purified CD34-Positive Cells Reconstitute Hematopoiesis

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Purpose: The objective of this study was to characterize CD34+ cell grafts, obtained using a novel technique, from children undergoing autologous bone marrow transplantation (BMT) for cancer therapy. In particular, we wanted to determine if the CD34+ marrow cell grafts generated hematopoietic reconstitution, since a positive result would motivate further development and use of this methodology.

Patients and Methods: This pilot feasibility clinical trial involved 13 patients ≤ 25 years of age with advanced solid tumors, including seven children with neuroblastoma. Harvested bone marrow underwent immunomagnetic CD34+ selection.

Results: In three of 13 enrolled patients, low purities of the CD34+ preparations disqualified the use of the CD34+ marrow grafts. Ten patients received myeloablative chemotherapy with etoposide, carboplatin, and cyclophosphamide, then were transplanted with CD34+ marrow grafts. In the 10 patients transplanted with CD34+-selected cells, the CD34+ cell purity (nucleated RBCs excluded) in the cell graft preparation was 91%, total cell recovery from the starting light-density cells 2.2%, CD34+ cell recovery 38%, colony-forming unit-granulocyte-macrophage (CFU-GM) recovery 23%, and estimated tumor-cell depletion 2.6 logs (medians). The CD34+ marrow grafts administered to these patients contained a median of 2.3 × 10⁸ nucleated cells, 1.4 × 10⁶ CD34+ cells, and 1.3 × 10⁵ CFU-GM per kilogram patient weight. Most patients experienced only the toxicities previously observed with this myeloactive chemotherapy regimen, although two unusual toxicities were observed. All 10 patients transplanted with CD34+ cell grafts engrafted.

Conclusions: The CD34+ purified grafts were enriched in stem/progenitor cells, with five of these 10 preparations containing ≥ 94% CD34+ cells. Engraftment with CD34+-purified cell grafts as pure as 99% confirms that autologous CD34+ cells, alone, are sufficient to provide hematopoietic rescue for myeloablated patients. The best purification results were obtained on small marrow harvests from patients with neuroblastoma. The engraftment of highly purified CD34+ cells obtained by this technology and the antitumor effect of the transplant, by which two of 10 poor prognosis patients remain clinically free of tumor, have stimulated further clinical trials.

J Clin Oncol 14:2224-2233. © 1996 by American Society of Clinical Oncology.

MOST CHILDHOOD solid cancers are sensitive to chemotherapy and radiotherapy, in that they initially respond with a clinical complete response (CR) or excellent partial response (PR). Nevertheless, the majority of cases of advanced (eg, metastatic) pediatric solid tumors eventually recur.[1-4] This is the cancer treatment situation — responsive tumors with high risk for recurrence — in which high-dose (myeloablative) chemotherapy has been used with autologous marrow rescue.[7] We developed a novel combination high-dose chemotherapy regimen for pediatric solid tumors.[8-10] We then desired a means to reduce the potential tumor-cell content of the autologous marrow to provide hematopoietic rescue for these patients, since tumor cells that contaminate the hematopoietic graft have been shown to contribute to tumor recurrence after transplant (in neuroblastoma).[11] Available methodologies in use to purge pediatric solid tumor cells from marrow include treatment of the autologous marrow graft with drugs or monoclonal antibodies.[7,12-14] Antineoplastic drug treatment of the marrow graft is toxic to the hematopoietic progenitors in the graft, and thereby extends the time to engraftment in heavily pretreated patients.[13] In addition, the efficacy of drug treatments for tumor purging has not been determined across the range of pediatric solid tumors. Finally, selective antitumor monoclonal antibodies have been available and clinically tested extensively only for neuroblastoma among pediatric solid tumors.[7] Thus, negative selection strategies face difficult limits to their clinical utility for purging of hematopoietic grafts from patients with pediatric solid tumors.

The combination of CD34 expression on lymphohematopoietic stem and progenitor cells with lack of expression on most cases of solid tumors suggests that immunoaffinity isolation (positive selection) of CD34+ cells can be used to reverse purge autologous marrow grafts for transplantation in a broad range of cancers.[15,16] Positive selection of CD34+ cells in clinical autologous bone marrow transplant (BMT) was first performed by Berenson et al.[17] They reported that hematopoiesis was reconstituted after transplantation of CD34+ cells, isolated using CD34 bio-

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Submitted September 25, 1995; accepted March 6, 1996.

Supported in part by Becton Dickinson Corp, San Jose, CA, and Baxter Healthcare Corp, Irvine, CA.

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0732-183X/96/1408-0006$3.00/0

tin-avidin immunoaffinity columns, in nine patients who had received myeloablative radiochemotherapy. Shpall et al[18] confirmed this with the report that CD34+ autologous bone marrow cells, isolated by a modification of the same methodology, produced engraftment in a larger series of patients with breast cancer. Several additional clinical trials using CD34+ cells from marrow and mobilized blood are now in progress with the goals of stem-cell enrichment and/or tumor depletion.[19]

The objective of this study was to characterize CD34+ cell grafts, obtained using a novel technique (Table 1), from children undergoing autologous BMT for cancer therapy. In particular, we wanted to determine if the CD34+ marrow cell grafts generated hematopoietic reconstitution, since a positive result would motivate further development and use of this methodology. This pilot feasibility clinical trial involved 13 patients.

PATIENTS AND METHODS

Patients

Thirteen patients ≤ 25 years of age with advanced solid tumors entered this study, after informed consent under a protocol approved by the Johns Hopkins Institutional Review Board and the United States Food and Drug Administration. Criteria for patient eligibility included adequate vital organ function, estimated survival greater than 8 weeks, Karnofsky score ≥ 60%, and bone marrow morphologically free from tumor at the time of bone marrow harvest. Since (1) chymopapain is used in this CD34+ cell purification procedure, (2) preexisting antibodies against chymopapain antibodies have been detected in approximately 1% of orthopedic patients evaluated for intradiscal injection of chymopapain for lumbar disc herniation, and (3) the low (≈ 0.5%) incidence of anaphylaxis following intradiscal chymopapain injection is reported to be minimized by excluding patients with positive tests for preexisting antichymopapain antibodies,[20] this study required the precaution that patients must have had a negative ChymoFAST test (Ignetics Inc, Palo Alto, CA) for antibody against chymopapain before bone marrow harvest.

The usual Pediatric Oncology Division practice for patients with advanced solid tumors is to attempt preautologous cytoreduction using multiple courses of dose-intensive chemotherapy, plus surgery and local radiation therapy directed at sites of initial or persistent tumors. In patients whose cancer progresses despite preautologous cytoreduction, transplant is generally not used. The 13 patients' pretransplant features are summarized in the results section.

Table 1. Outline of CD34+ Selection, Cryopreservation, and Infusion Procedures

- Centrifugal buffy-coat leukocyte preparation.
- Ficoll-Hypaque centrifugal density gradient. Centrifugal washes of marrow mononuclear cells.
- Incubate with CD34 (My10) monoclonal antibody (IgG_1). Centrifugal washes.
- Incubate sensitized cells with sheep antimouse IgG_1-coated immunomagnetic microspheres. Magnetic washes on Isolex to remove CD34- cells.
- Clip microspheres from CD34+ cells using chymopapain. Remove free microspheres using Isolex. Centrifugal washes of cells. Remove any residual microspheres with a second magnet.
- Analyze and cryopreserve CD34+ graft.
- Myeloablative transplant chemotherapy:
  Etoposide: 2,400 mg/m² total dose;
  Carboplatin: 2,175 mg/m² total dose;
  Cyclophosphamide: 120 mg/kg total dose (with mesna).
- Bone marrow graft infusion on transplant day 0, 48 hours after the final dose of myeloablative chemotherapy. To minimize the chance of anaphylactic reaction against trace residual amounts of chymopapain or antibody, patients received:
  Dexamethasone,
  Diphenhydramine, and
  Ranitidine.

Bone Marrow Harvest and Processing

Bone marrow collection was performed following standard procedures. Sufficient marrow was aspirated to obtain greater than 2×10^8 nucleated marrow cells per kilogram patient weight. Aspirated marrow diluted in RPMI 1640 (BioWhittaker, Walkersville, MD) that contained preservative-free heparin was filtered through a series of filters of decreasing pore size (Baxter-Fenwal, Deerfield, IL) to remove particles and cell clumps. A minimum of 0.5×10^8 nucleated marrow cells per kilogram was cryopreserved as an unpurified back-up marrow.[3]

The remainder of the harvested marrow underwent immunomagnetic CD34+ selection (Table 1). First, a buffy coat was prepared by centrifugation using a COBE 2991 (Cobe, Lakewood, CO) cell processor (patients no. 1, 3, 5, 7, 9, 10, and 13), or for samples with low total cell numbers, nonautomated centrifugation in a blood transfer pack (Baxter-Fenwal; patients no. 2, 4, 6, 8, 11, and 12). This preparation was then further enriched for marrow mononuclear cells by Ficoll-Hypaque (BioWhittaker) density gradient centrifugation on the COBE 2991. To sensitize target CD34+ cells with monoclonal antibody, the marrow mononuclear cells (up to 5×10^8 cells/mL) RPMI 1640 that contained 1% human serum albumin (Baxter Healthcare, Glendale, CA) and 0.5% human immune globulin (Sandoz, East Hanover, NJ) were incubated for 30 minutes at 4°C in a blood transfer pack with My10 antibody (0.5 μg hematopoietic progenitor cell antigen-1 [HPCA-1] antibody preparation/10^8 cells; Becton Dickinson Immunocytometry Systems, San Jose, CA) and then washed with RPMI 1640 that contained 1% human serum albumin to remove free antibody. For patient no. 1, this washing was done by centrifugation using the COBE cell processor; after low purity of CD34+ cells was obtained in this first patient, washing was performed by a standard centrifugal wash in 50-mL conical centrifuge tubes for the remaining patients. The cells were then incubated (30 minutes at 4°C) with sheep antimouse immunoglobulin G_1 (IgG_1)-coated paramagnetic microspheres (two cells to one bead ratio; Dynal, Lake Success, NY). Unbound CD34- cells were removed by collecting the microsphere-cell rosettes (along with the free microspheres) using the prototype Isolex device (a prototype magnetic cell separation device that consists of an array of permanent magnets and a customized sterile plastic disposable chamber) and procedure (Baxter Healthcare Immunotherapy Division, Irvine, CA),[21,22] followed by four 50-mL washes using the prototype Isolex device. Incubation (15 minutes at room temperature with end-over-end rotation) with chymopapain (200 U/mL; Chymodiactin; Sovia Pharmaceuticals, Lincolnshire, IL) was performed to release microspheres and antibodies from rosetted CD34+ cells. The free micro-

spheres were then removed by passage over the prototype Isolex device and the CD34+ cells were collected in a blood transfer pack. CD34+ cells were concentrated by centrifugation of the blood transfer pack. Cells were transferred to a 50-mL conical centrifuge tube for a wash using RPMI 1640 that contained 1% human serum albumin, then resuspended to 10 mL and placed on a magnet to remove residual beads. Cryopreservation of the purified CD34+ cells was performed by standard controlled-rate freezing (Cryomed; Forma, Marietta, OH) in a plastic freeze bag (Baxter-Fenwal) in RPMI 1640 that contained 10% dimethylsulfoxide and 20% autologous patient plasma. This material was finally stored under liquid nitrogen until use. Before cryopreservation, a small aliquot of the purified cell preparation was withheld for analysis.

Analysis of CD34+-Purified Bone Marrow Specimens

The percent CD34+ cells present in the purified cell preparation was determined using flow cytometry with the anti-HPCA-2 antibody (Becton Dickinson), which recognizes a chymopapain-resistant epitope of the CD34 molecule.[28,29]

If the purified cell preparation contained ≥ 40% CD34+ cells after exclusion of nucleated RBCs, and if the estimated number of CD34+ cells in the purified CD34+ cell fraction was ≥ 10^6 cells/kg, the CD34+ cell fraction was thawed and administered intravenously (IV) as the transplant graft on day 0. The initial protocol study design and informed consent specified that the patient would not be exposed to the potential risks of the experimental CD34+ cell preparation as the transplant graft unless both of these criteria were met, but instead would be considered for BMT using the unprocessed, cryopreserved back-up marrow preparation. All patients met the criterion for total numbers of CD34+ cells obtained. However, in three cases (patients no. 1, 10, and 13), the purified CD34+ cell preparation did not meet the ≥ 40% purity criterion. For this reason, two of these three patients (no. 1 and 13) received the back-up marrow preparation instead of the purified marrow preparation. Patient no. 10 was never transplanted because his tumor progressed during pretransplant therapy.

Myeloablative Chemotherapeutic Regimen

Patients received the following myeloablative chemotherapeutic regimen before bone marrow rescue: etoposide 2,400 mg/m² (800 mg/m²/d by IV continuous infusion on days −6 to −4), carboplatin 2,175 mg/m² (725 mg/m²/d IV over 1 hour on days −6 to −4), and cyclophosphamide 120 mg/kg (60 mg/kg/d IV over 1 hour on days −3 and −2). Mesna 12 mg/kg by IV push was given at 0, 3, and 6 hours after cyclophosphamide. The bone marrow graft was infused on day 0, 48 hours after the final dose of cytoreductive chemotherapy. To minimize the chance of anaphylactic reaction against trace residual amounts of chymopapain or antibody that might be present in the CD34+ selected marrow graft, patients received the following medications: dexamethasone 0.1 mg/kg per dose IV every 6 hours for a total of eight doses beginning 12 hours before the CD34+ cell graft infusion, benadryl 0.5 mg/kg per dose IV every 6 hours for a total of eight doses beginning 12 hours before the graft infusion, and ranitidine 1 mg/kg per dose IV every 8 hours for a total of five doses beginning 16 hours before the graft infusion.

Care After BMT

Patients were cared for using Johns Hopkins Hospital pediatric BMT policies and guidelines. In most cases, platelet products were transfused when the platelet count decreased to less than 20,000/μL, and RBCs (packed cells) were transfused to maintain a hematocrit level greater than 20 to 30. Hematopoietic growth factors were not used after BMT.

RESULTS

Patient Characteristics, Pretransplant Therapy, and Response

Seven of 13 patients, with an age range of 1 to 5 years, had neuroblastoma. Patients no. 6, 8, 9, 11, and 12 had Pediatric Oncology Group (POG) stage D (Evans stage IV/International Neuroblastoma Staging System [INSS] stage 4[27]) neuroblastoma with metastatic sites including bones. Patient no. 2 was classified as POG stage C with a large adrenal neuroblastoma with local extension and malignant ascites. He qualified for BMT because of elevated *N-myc* gene copy number (n = 326) in his tumor specimen[30,31] and malignant ascites. Patient no. 4 had POG stage B adrenal neuroblastoma, but qualified for BMT because of elevated *N-myc* gene copy number (n = 94) in his tumor specimen. In all of these patients with neuroblastoma, BMT was planned from early in initial treatment and was performed after completion of five to 10 courses of chemotherapy plus second-look surgery and irradiation to sites of initial and remaining clinically detectable tumors. After receiving all pretransplant multimodal therapy by the time of BMT, patients no. 2 and 4 were in clinical CR and had no microscopic disease identified at second-look surgery. Patient no. 3 had no detectable tumor by noninvasive studies, but had microscopic neuroblastoma at the second-look surgical margins. Patients no. 9 and 11 also had no detectable tumor by noninvasive studies, but had microscopic adrenal neuroblastoma (resected at second-look surgery) and residual abnormalities on technetium bone scan. Thus, patients no. 2, 4, 5, 9, and 11 were in CR at the time of BMT by the INSS definitions of response.[27] Patients no. 6 and 12 had only PRs by the INSS staging criteria immediately before transplant. In all patients with neuroblastoma, sites of initial bulk disease and detectable disease were irradiated before BMT.

Patients no. 3 and 5 were young adults with germ cell tumors. In patient no. 3, the tumor had recurred after two chemotherapy regimens. He had a clinical CR in that his tumors again shrunk with additional cytoreductive chemotherapy, and he received radiation therapy to remaining clinically evident tumor sites before BMT. In patient no. 5, the cancer recurred, with an increased human chorionic gonadotropin levels less than 2 months after he had received four courses of chemotherapy. He was transplanted, in progressive disease status, with growing metastatic tumor nodules, which were not irradiated.

Patient no. 1 was an adolescent with tibial osteosarcoma treated with limb-sparing surgery and adjuvant chemotherapy. Two years after initial diagnosis, a pulmonary metastasis was detected radiologically. Surgical wedge resection removed this single detectable metastatic lesion before BMT, which made his tumor status clinical CR.

Patient no. 7 was a young adult referred for BMT with malignant epithelial thymoma that had recurred after surgery, then had responded to chemotherapy, but regrew and extended into the left lung. After the thymoma again responded to additional chemotherapy, BMT was performed at the time of a clinical CR. After BMT, he was treated with radiation therapy to the left hemithorax (see later).

Patient no. 10 was an adolescent with widely metastatic prostatic rhabdomyosarcoma. His marrow became morphologically free of tumor for a brief time during chemotherapy, which allowed bone marrow to be harvested; however, the marrow was hypocellular. This patient experienced tumor progression during chemotherapy before BMT could be attempted.

Patient no. 13 was an 8-year-old girl with undifferentiated embryonal sarcoma of the liver and peritoneal metastatic implants. The tumor had an excellent PR to chemotherapy, but second-look surgery showed residual peritoneal metastases with a tumor nodule near the dome of the bladder. She received additional chemotherapy and, after bone marrow harvest, abdominal radiation therapy by external beam and phosphorous-32 instillation after bone marrow harvest but before BMT.

Of the 13 patients enrolled onto this protocol, 10 were transplanted with CD34+ marrow grafts. In the other three patients (no. 1, 10, and 13), low purities (< 40% CD34+ cells) of the CD34+ preparations disqualified the use of the CD34+ marrow grafts, based on the study design (see Methods). In two of these three patients, BMT was performed using the back-up marrow to provide hematopoietic rescue. In one patient, tumor progression prevented BMT (Tables 2 and 3).

In all seven patients with advanced neuroblastoma, BMT was planned from the time of initial diagnosis, as intensive consolidation therapy after initial multiagent chemotherapy, second-look surgery, and local radiation therapy. Two of the other three patients transplanted with CD34+ marrow grafts (patients no. 3 and 7) were not referred for BMT until their tumors had recurred twice. Patient no. 3 was transplanted when his tumor was progressing after the initial chemotherapy regimen. All 10

Table 2. Marrow Graft Cell Processing Results*

Unique Patient No.	Unprocessed Bone Marrow Harvest (nucleated cells x 10^8/kg)	Starting Light-Density Cell Preparation After Ficoll-Hypaque Density Gradient		Purified CD34+ Cell Preparation							
		Nucleated Cells x 10^8/kg	% CD34+	Nucleated Cells x 10^8/kg	% of Nucleated Cells in Starting Light-Density Nucleated Cell Preparation	% CD34+	% CD34+ (nucleated RBCs excluded)	% of CD34+ Cells in Starting Light-Density Nucleated Cell Preparation	CD34+ Cells x 10^8/kg	% of CFU-GMs in Starting Light-Density Nucleated Cell Preparation	CFU-GMs x 10^6/kg
1*	4.9	0.4	2.5	2.5	6.7	12.3	12.6	33	0.3	12	0.5
2	6.4	1.4	4.5	2.7	1.9	98.4	98.6	40	2.6	76	11.4
3	3.6	0.4	2.0	0.3	0.9	67.4	84.7	20	0.2	22	0.8
4	4.9	1.4	3.1	10.3	7.4	17	38.0	40	1.8	70	3.3
5	4.8	0.7	2.8	1.0	1.3	63.7	80.9	20	0.6	73	9.0
6	4.5	0.8	5.6	3.1	4.0	19.0	28.5	14	0.6	29	1.3
7	4.2	0.4	4.6	2.3	6.4	86.2	88.0	37	0.9	6	0.3
8	7.0	1.4	6.5	5.0	3.5	93.9	97.7	51	4.7	18	1.8
9	4.4	0.7	6.0	1.6	2.4	98.3	98.3	39	1.6	14	0.7
10*	4.0	0.7	2.2	0.9	1.3	17.5	19.2	11	0.2	2	0.1
11	6.8	1.7	4.2	2.4	1.4	94.3	97.4	31	2.2	11	1.0
12	4.8	1.3	2.2	1.2	0.9	96.9	96.9	40	1.2	18	1.2
13*	4.0	1.9	3.4	11.3	6.0	14.2	20.7	23	1.6	16	2.4
Mean	5.0	1.0	3.8	3.4	3.4	58.3	68.4	32	1.4	29	2.6
Median	4.8	0.9	3.4	2.4	2.9	59.5	79.7	33	1.3	15	1.3
Mean, excluding patients 1, 10, and 13	5.2	1.0	4.2	3.0	3.0	67.5	83.1	35	1.6	35	3.1
Median, excluding patients 1, 10, and 13	4.8	1.0	4.4	2.3	2.2	80.6	90.8	38	1.4	23	1.3

NOTE. Values have been rounded. Means and medians were calculated before rounding of the primary measured values.
* Patients not transplanted with CD34+ grafts.

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Table 1. Clinical Results of Autologous Marrow Transplants

Unique Patient No.	Days From BMT Graft Infusion Until: WBC Count ≥ 1,000/μL	ANC ≥ 200/μL	ANC ≥ 500/μL	Platelets ≥ 50,000/μL	Last Platelet Transfusion	Hospital Discharge	Estimated Tumor-Cell Depletion (logs)	Time to Tumor Progression or Recurrence (months after CD34⁺ BMT)	Survival (months after CD34⁺ BMT)
1*	42	42	49	63	36	30	1.2	16	26
2	40	44	49	49	33	34	3.4	37+	37+
3	30	26	28	34	22	31	2.5	5	38+
4	43	43	55	33	32	37	1.2	33+	33+
5	31	29	34	29	23	47	2.3	1	12
6	32	32	38	52	32	26	1.5	5	6
7	27	24	31	31	24	27	1.3	25	28+
8	19	16	26	24	21	28	2.6	5	5
9	30	25	30	53	53	36	3.4	20	24+
10*	No BMT	No BMT	No BMT	No BMT	No BMT	No BMT	2.0	No BMT	No BMT
11	33	19	36	36	28	20	3.1	7	14
12	51	40	57	61	57	40	3.5	4	16+
13*	42	42	42	103	67	45	1.3	5	8
Mean†	37	33	41	46	33	34	2.3	14	23
Median†	38	34	40	42	32	34	2.3	6	20
Mean, excluding patients 1, 10, and 13	36	31	40	41	33	32	2.5	16	21
Median, excluding patients 1, 10, and 13	32	30	37	35	32	33	2.6	6	20

NOTE. Values have been rounded. Means and medians were calculated before rounding of the primary measured values. Data as of May 1996.
* Patients not transplanted with CD34⁺ grafts.
† Values (except for column 8: estimated tumor-cell depletion) exclude patient no. 10, who did not undergo BMT.

Delayed

patients transplanted with CD34⁺ marrow grafts were treated heavily with chemotherapy before autologous marrow harvest, with from four to 22 cycles of multiagent chemotherapy, which included from three to eight antineoplastic drugs. Eight of these 10 patients received local radiation therapy 1 to 3 weeks before BMT; patient no. 7 received radiation after BMT, and patient no. 5 received no radiation therapy.

Bone Marrow Processing and Cell Purification Results

Bone marrow graft processing required approximately 7 hours from the time of receipt of the harvested marrow to cryopreservation. Approximately 4 hours of this time was spent performing the CD34⁺ selection procedure itself. Cell processing results are listed in Table 2. Total bone marrow nucleated cells harvested ranged from 4.7 to 33.5 × 10^9 cells per patient (largely as a function of patient size), and from 3.6 to 7.0 × 10^8 cells/kg patient weight (median, 4.3 × 10^8 cells/kg). Ficoll-Hypaque density gradient centrifugation reduced the preparations to 0.8 to 5.2 × 10^9 cells per patient and 0.4 to 1.9 × 10^8 cells/kg patient weight (median, 0.9 × 10^8 cells/kg). These light-density cell preparations were the starting cells for the CD34⁺ immunoaffinity purifications, and they contained from 2.0% to 6.5% CD34⁺ cells (median, 3.6%) and 34 to 174 colony-forming units–granulocyte-macrophage (CFU-GM)/10^5 nucleated cells (median, 69; mean, 82).

After CD34⁺ selection with immunomagnetic microspheres, a median of 2.9% (mean, 3.4%; range, 0.9% to 7.4%) of the starting light-density nucleated cells were recovered in the CD34⁺ cell preparation (total cell recovery). These 13 CD34⁺-purified cell preparations contained a median purity of 80% (mean, 68%; range, 15% to 99%) CD34⁺ cells, if nucleated RBCs were excluded from the analysis by flow-cytometric gating, and 60% (mean, 55%; range, 12% to 99%) if nucleated RBCs were included. The median percent recovery of CD34⁺ cells from the starting light-density cell preparation (CD34⁺ cell recovery) was 33% (mean, 32%; range, 11% to 51%). Median recovery of CFU-GM in the starting light-density cells was 18% (mean, 29%; range, 2% to 76%).

In three of 13 patients, the CD34⁺-purified cell preparations contained less than 40% CD34⁺ cells after exclusion of nucleated RBCs, which disqualified use of these CD34⁺ cell preparations as their BMT grafts (see Methods). Two of these three patients (no. 1 and 13) underwent BMT, but received their unpurged back-up marrow preparations instead of the CD34⁺-selected cells. The third patient (no. 10) was not transplanted due to tumor pro-

gression. In the 10 patients who were transplanted with CD34+ selected cells, the median CD34+ cell purity (nucleated RBCs excluded) was 91% (mean, 83%; range, 48% to 99%), the median total cell recovery from the starting light-density cells was 2.2% (mean, 3.0%; range, 0.9% to 7.4%), the median CD34+ cell recovery was 38% (mean, 35%; range, 14% to 51%), and the median recovery of CFU-GM was 23% (mean, 35%; range, 6% to 76%). The CD34+ marrow grafts administered to these 10 patients contained a median of 2.3×10^8 nucleated cells (mean, 3.0; range, 0.3 to 10.3), 1.4×10^6 CD34+ cells (mean, 1.6; range, 0.6 to 4.7), and 1.3×10^5 CFU-GM (mean, 3.1; range, 0.3 to 11.6) per kilogram patient weight (Table 2).

Toxicity

None of the 13 patients had positive ChymoFAST tests for preexisting antibody against chymopapain. All 10 patients transplanted with CD34+ cell grafts tolerated infusion of CD34+ cells without bradycardia, hypotension, hypertension, or signs of anaphylaxis. These patients required close monitoring of electrolytes and infusions of potassium, phosphate, magnesium, and bicarbonate to compensate for renal wasting for several days after high-dose carboplatin. Two patients had transient hypertension, and in two patients the serum creatinine level transiently increased to 3.0 mg/dL, but returned to less than 1.5 mg/dL by hospital discharge. No patient developed clinical renal failure. One patient had transient hemorrhagic cystitis, attributed to cyclophosphamide, from days 10 to 13 after transplant.

All 10 patients experienced mucositis and routinely received IV alimentation and IV opiate analgesia. Frequent minor problems associated with the preparative chemotherapy regimen included transient elevations in bilirubin and hepatic enzymes and tinnitus with high-frequency sensorineural hearing loss. All patients had profound myelosuppression. Associated fevers were treated empirically with IV antibiotics. Only patients no. 8 and 9 had positive blood cultures (*Acinetobacter lwoffi* and *Klebsiella pneumoniae*). In patient no. 2, cytomegalovirus was cultured from urine, and this was temporally associated with prolonged time to hematopoietic engraftment. One patient had maxillary sinusitis diagnosed by computed tomography, and two patients had perirectal erythema, but these suspected infections did not result in positive blood cultures or clinical progression. No patients had blood cultures positive for fungi.

Unexpectedly, on day 1 after transplant, patient no. 5 developed acute paraplegia, with hypesthesia at the level of L4-L5. Extensive neurologic evaluation, including lumbar puncture and magnetic resonance imaging, failed to explain this permanent transverse myelitis. Cisplatin and carboplatin both have neurotoxicity in high doses.[30,31] Transverse myelitis is a reported complication of intradiscal administration of chymopapain,[32,33] but in this protocol involving ex vivo use of chymopapain with only trace residual amounts infused IV to the patient, it appears unlikely that chymopapain caused this problem. Tumor involvement of the spinal cord was suspected, but was not proven, and autopsy was declined by the family of this patient, who died of tumor progression in other sites.

In summary, most patients experienced only the transient toxicities previously observed with this preparative chemotherapy regimen, including myeloablation, mucositis, proximal tubular renal electrolyte wasting, hemorrhagic cystitis, high-frequency sensorineural hearing loss, and asymptomatic hepatic enzyme elevation.[5-8] One patient developed unexplained transverse myelitis. There were no episodes of veno-occlusive disease of the liver or pneumonitis, and no patient developed fatal toxicities in the immediate peritransplant period.

Hematopoietic Engraftment

All transplanted patients engrafted (Table 3). In the 10 patients who received CD34+ marrow grafts, the median time until posttransplant recovery of the WBC count to $\geq 1{,}000/\mu L$ was 32 days (mean, 36; range, 19 to 51). The median times to absolute neutrophil count $\geq 200/\mu L$ and $500/\mu L$ also ranged widely, with a median of 30 (mean, 31; range, 16 to 46) and 37 (mean, 40; range, 26 to 55) days, respectively. The platelet count recovered to $\geq 50{,}000/\mu L$ by a median of 35 days (mean, 41; range, 29 to 61) posttransplant, and the last platelet transfusion was at a median of day 32 (mean, 35; range, 21 to 57). Because of historical variations in the medical reasons for RBC transfusions, it was decided prospectively not to determine time to erythroid recovery. As can be seen from Table 3, there was no correlation between time to engraftment and numbers of infused nucleated cells, CD34+ cells, or CFU-GM. The median duration of hospitalization was 33 days (mean, 32; range, 20 to 47) posttransplant. Table 3 also lists the clinical results for the two patients transplanted with unprocessed back-up marrow graft preparations (patient no. 1 and 13) for comparison, and the means and medians are listed for the entire group of 12 transplanted patients, as well as for the 10 patients actually transplanted with CD34+ grafts.

Patient no. 7 experienced an unusual hematopoietic problem. At 3 to 4 months posttransplant during the administration of adjuvant radiation to a wide field including the initial extent of his mediastinal thymoma, his hemato-

crit level and platelet count decreased. He eventually became dependent on approximately weekly transfusions of RBCs and platelets, although his WBC count did not decrease to dangerous levels. Bone marrow aspirates and biopsies repeatedly showed decreased erythroid precursors and megakaryocytes. Extensive evaluations did not find infections (including parvovirus) or recurrent cancer. Antibodies could not be detected against RBCs or platelets. As a child, more than a decade before being diagnosed with thymoma, this patient had had an episode of idiopathic thrombocytopenia that responsed completely to brief treatment with corticosteroids. Eleven months posttransplant, after several months of observation and unsuccessful treatment with IV immunoglobulin and corticosteroids, patient no. 7 received an unprocessed marrow back-up graft. No response was detected in blood cell counts or marrow aspirate morphology by 2 months after the back-up marrow infusion, and the patient still required packed RBC and platelet support. Since more than 1 year ago, this patient has carried the diagnosis of thymoma-associated autoimmune thrombocytopenia/anemia.[34] He is now being treated with cyclosporine, with a increase in platelet and RBCs counts and elimination of transfusion requirements.

Tumor Progression and Patient Survival

Of 10 patients who received CD34$^+$ marrow grafts, four (no. 5, 6, 8, and 11) have died, all with tumor progression, and four are alive with tumor present (no. 3, 7, 9, and 12) (Table 3). Currently, the median survival time for this group of patients transplanted with CD34$^+$ marrow grafts is 20 months (mean, 20; range, 5 to 37+) posttransplant. Four of these 10 patients experienced ≥ 20 months from transplant to tumor recurrence. The three patients with neuroblastoma with no detectable tumor for ≥ 20 months posttransplant received BMT as intensive consolidation therapy at the end of their initial treatment periods, and received a minimum of five cycles of standard-dose chemotherapy to achieve a CR (patients no. 2, 4, and 9). All three patients had gross removal of accessible tumor before BMT, but patient no. 9 had extensive bony metastases, which could not be removed. In these patients, all detectable sites of persistent or initial neuroblastoma, including treatable bony sites, were irradiated. Patient no. 9 had a long tumor-free interval (neuroblastoma recurrence 20 months posttransplant), despite the fact that she had extensive bony metastases at diagnosis. Prior studies report rare survivors, even with BMT, for patients with bony metastases.[35] Two patients (no. 2 and 4) do not yet have clinical evidence of recurrent cancer, at 33 and 37 months posttransplant. The pre-BMT treatment, the BMT preparative regimen, and the efficacy of marrow graft purging may all contribute to the prolonged survival of these patients, but the results support further investigation of this approach in neuroblastoma.

In retrospect, it would have been interesting to have performed direct assays for residual tumor cells to assess the efficacy of reverse purging during the CD34$^+$ graft purifications. However, even today, direct assays for small numbers of residual tumor cells are unavailable for most of these pediatric solid tumors. Even where available, the sensitivity of assays for minimal residual neuroblastoma and Ewing's/primitive neuroectodermal tumors, these assays are already near their detection limits (sensitivity, ≈ 10^{-5} to 10^{-6}) in patients with no evident tumor by routine clinical tests.[12,14,16,36] Morphologic analysis of marrow should detect approximately 1% tumor contamination (10^{-2}). If the tumor purging method then gives just 1 to 2 logs of further tumor depletion of the graft preparation, the tumor detection method would be at or beyond its limits and might miss fairly large amounts of residual tumor in the graft. Thus, a surrogate assay that depends on more easily measured events would be useful.

To model the effect of each patient's CD34$^+$ cell purification on the tumor content of that patient's autograft, we assumed that the patient's tumor cells behaved as other CD34$^+$ cells during the CD34$^+$ selection. Thus, the reduction in tumor-cell number would be equivalent to the reduction in CD34$^+$ cell number. This allowed estimation of the tumor-cell depletion (reverse purging effect) from the starting light-density cells to the CD34$^+$-selected graft in each patient, by use of the following formula[14,16]: estimated tumor-cell depletion (logs) = log (no. CD34$^+$ cells in the starting mononuclear cell preparation/no. of CD34$^+$ cells in the final CD34$^+$ graft preparation). The calculated values obtained are listed in Table 3; note that these calculated values are potential surrogates for extent of tumor purging, but do not reflect direct measurements of tumor cells present in the cell preparations. The calculated median tumor-cell depletion was 2.6 logs (mean, 2.5; range, 1.2 to 3.5) for the 10 patients transplanted with CD34$^+$ marrow grafts, and 2.3 logs (mean, 2.3; range, 1.2 to 3.5) for all 13 patients.

Immune Function After BMT

Immune function has been assessed in the three neuroblastoma patients with ≥ 20-month tumor-free intervals posttransplant. At time points greater than 1 year after BMT, all three patients have developed antibody titers against diptheria and tetanus toxoids, to which they had been immunized before their tumors were diagnosed. Two of the three have already been immunized with, and

developed antibody titers against recombinant hepatitis B vaccine, in the absence of preexisting antibody or infection. In addition, these patients have had no unusual infectious problems.

DISCUSSION

The main objective of this clinical study was to test whether autologous CD34+ marrow cells, positively selected with My10 (CD34) monoclonal antibody and immunomagnetic microspheres and released by chymopapain, restored lymphohematopoiesis in children and young adults with advanced solid cancers. The CD34+ selection procedure, listed in Table 1, was based on a research laboratory procedure,[38] which was scaled up as a prototype for clinical use.[22,23] After myeloablative chemotherapy, 10 patients were transplanted with CD34+ autologous marrow grafts. The CD34+-purified grafts of these 10 patients were enriched in stem/progenitor cells, with five of these 10 preparations containing ≥ 94% CD34+ cells. Hematopoietic reconstitution was observed in all of these patients. Engraftment with CD34+-purified cell grafts as pure as 99% confirms that autologous CD34+ cells, alone, are sufficient to provide hematopoietic rescue for myeloablated patients.

On the other hand, hematopoietic engraftment following these transplants of CD34+ cell grafts required about 5 weeks, approximately 1 week longer than in the prior clinical trial in which heavily treated patients with advanced pediatric solid tumors received whole (unprocessed) bone marrow.[5,40] No hematopoietic growth factors were administered after BMT in either of these trials. Thus, it cannot be excluded that the CD34+ cell purification procedure resulted in some loss of or injury to stem/progenitor cells that contributed to engraftment delay. However, the range of the times to hematopoietic recovery in both studies were large, and the number of patients small. In addition, prior larger BMT studies in neuroblastoma have reported similar times to engraftment and concluded that pretransplant chemotherapy was probably responsible.[2,41] Finally, in the two patients of the study reported here who were transplanted with unprocessed back-up marrow preparations instead of the CD34+ preparations (because the purity of their CD34+ preparations was < 40%; see Methods and Results), the times to hematopoietic recovery were prolonged: times to a neutrophil count of 500/μL and platelet count of 50,000/μL were 49 and 63 days, respectively, in patient no. 1, and 42 and 103 days, respectively, in patient no. 13 (Table 3). Thus, it is possible that this was a group of patients who (on average) would have been slow to engraft even without CD34+ cell purification, possibly due to intensive chemotherapy treatment before bone marrow harvest and transplant. Whether this CD34+ cell purification procedure affects time to hematopoietic recovery could be tested in a concurrent randomized clinical trial, using patients who received unprocessed marrow as a control group. Preliminary results of our current trial (in similar patients with pediatric solid tumors) of transplantation of CD34+ cells mobilized from blood, with or without marrow CD34+ cells, indicate that when granulocyte colony-stimulating factor (G-CSF) is administered post-BMT, CD34+ cell grafts purified by this method engraft promptly.[42]

Engraftment of autologous marrow grafts may demonstrate the presence of adequate numbers and function merely of progenitor cells. After autologous transplant, long-term hematopoiesis may be due, not to stem cells from the graft, but to endogenous stem cells that survived the myeloablative preparative regimen in the host. Thus, autologous transplants with genetically marked purified CD34+ cells[11] or, easier, allogeneic transplants of purified CD34+ cell grafts will need to be assessed to prove whether long-term lymphohematopoiesis derives from the grafted CD34+ cells.

In three of 13 patients enrolled onto this study, purities of the CD34+ graft preparations as low as 12% disqualified the use of the CD34+ marrow grafts. The best purification results were obtained on small marrow harvests from patients with neuroblastoma. The capacity of the CD34+ cell selection device has been increased with the Isolex system now used for wider clinical trials. In addition, the Isolex system has been further engineered to be faster and require less technician input.[42]

There were two unusual, severe toxic events in this trial—transverse myelitis and chronic anemia/thrombocytopenia. Other observed toxicities appeared to be directly attributable to the chemotherapy preparative regimen. Ongoing, larger clinical trials will provide further information on whether these toxicities are rare problems in these two individual patients or are due to the CD34+ cell selection.

There is direct evidence that neuroblastoma cells present in patients' bone marrow grafts can contribute to relapse.[11] To model the effect of each patient's CD34+ cell purification on the tumor content of that patient's CD34+-selected autograft, it was assumed that the patient's tumor cells behaved as typical CD34+ cells during the CD34+ selection, and copurified with the normal CD34+ cells.[38,39] Using this model, we calculated an estimate of the tumor-cell depletion (reverse purging effect) predicted by the CD34+ purification results in each patient. As high as 3.5-log tumor-cell depletions were predicted by this model in purifications with high CD34+ cell purities in the grafts.

However, the calculated tumor-cell depletion was not this high in other cases (Table 3), and direct measurements of tumor-cell content should also be performed in future studies.

If CD34+ cell purification technology can be improved to obtain CD34+ cells in greater than 90% purity and greater than 3-log tumor-cell depletion routinely, the reverse purging effect would be comparable to the effect of negative selection purging technologies, such as drug or antibody plus complement, as reported in clinical studies.[12-14] Clearly, experimental measurements of tumor cells in the graft preparations would be preferred to modeling. This is being done in ongoing clinical trials, but with the limitation that most of the available methods for detection of minimal residual disease are near their limits of sensitivity in quantitating tumor content of marrow or blood specimens from patients at the time of stem-cell harvest.[12,14,16-18] Thus, precise quantitation of the reverse purging effect of CD34+ purification will be difficult in patients with low numbers of tumor cells in the marrow (or blood) at the time of harvest. If more tumor-cell depletion is needed than can be reproducibly obtained by a single CD34+ purification of the graft, methods are now available[13,14] that would permit repeated (sequential) CD34+ purification of the graft to multiply the reverse purging effect. Other possibilities for further depleting tumor cells include combining positive with negative separation[19] and culturing of the CD34+ selected cells under conditions that favor proliferation of stem/progenitor cells, but death of tumor cells.[19]

The engraftment of highly purified CD34+ cells obtained by this technology and the antitumor effect of the transplant, by which two of 10 poor-prognosis patients remain clinically free of tumor, have stimulated our current study in advanced pediatric solid tumors. To speed engraftment and decrease tumor contamination of the grafts, this trial involves use of an improved procedure and device for the immunomagnetic CD34+ cell selection, mobilized blood as the starting material for the grafts, and G-CSF treatment after transplant.[3]

ACKNOWLEDGMENT

We thank the patients and families who participated in this study, and the physicians and staff who provided superb medical and supportive care.

Becton Dickinson Corp and Baxter HealthCare Corp provided partial research funding for this clinical trial. The Johns Hopkins University holds patents on CD34 monoclonal antibodies and related inventions. Dr Civin is entitled to a share of the sales royalty received by the University under licensing agreements between the University and these companies. Dr Civin has also served as a consultant for these companies. The terms of these arrangements have been reviewed and approved by the University in accordance with its conflict of interest policies.

REFERENCES

1. Bonilla MA, Gaynon PV: Clinical progress in neuroblastoma. Cancer Invest 12:644-653, 1994

2. Graham-Pole J, Casper J, Elfenbein G: High-dose chemoradiotherapy supported by marrow infusions for advanced neuroblastoma: A Pediatric Oncology Group study. J Clin Oncol 9:152-158, 1991

3. Philip T, Zucker JM, Bernard JL: Improved survival at 2 and 5 years in the LMCE1 unselected group of 72 children with stage IV neuroblastoma older than 1 year of age at diagnosis: Is cure possible in a small subgroup? J Clin Oncol 9:1037-1044, 1991

4. Hartmann O, Beaujean F, Benjamin F, et al: High-dose busulfan and cyclophosphamide with autologous bone marrow transplantation support in advanced malignancies in children: A phase II study. J Clin Oncol 4:1804-1810, 1986

5. Graham ML, Yeager AM, Leventhal BG, et al: Treatment of recurrent refractory pediatric solid tumors with high-dose busulfan and cyclophosphamide followed by autologous bone marrow rescue. J Clin Oncol 10:1857-1864, 1992

6. Allen JC, Helson L: High-dose cyclophosphamide chemotherapy for recurrent CNS tumors in children. J Neurosurg 55:749-756, 1981

7. Seeger R, Reynolds C: Treatment of high-risk solid tumors of childhood with intensive therapy and autologous bone marrow transplantation. Pediatr Clin North Am 38:393-424, 1991

8. Wiley JM, Leventhal BG, Fresia A, et al: High dose carboplatin (CBDCA), etoposide (VP-16) and cyclophosphamide (CY) with autologous bone marrow rescue (ABMR) in children and young adults with solid tumors: Results of a phase I study. Proc Am Soc Clin Oncol 12:419a, 1993 (abstr)

9. Wiley J, Fresia A, Strauss L, et al: High dose carboplatin (CBDCA), etoposide (VP-16) and cyclophosphamide (CY) with autologous bone marrow rescue (ABMR) in children and young adults with refractory solid or brain tumors. Proc Am Soc Clin Oncol 11:374a, 1993 (abstr)

10. Wiley JM, Strauss LC, Fresia A, et al: High dose chemotherapy and autologous bone marrow rescue in poor risk sarcoma patients. A report of a pilot study in pediatric sarcomas. Proceedings of the Sixth International Symposium on Autologous Bone Marrow Transplantation, Arlington, VA, 1993, pp 199-204

11. Rill DR, Santana VM, Roberts WM, et al: Direct demonstration that autologous bone marrow transplantation for solid tumors can return a multiplicity of tumorigenic cells. Blood 84:380-383, 1994

12. Gribben JG, Freedman AS, Neuberg D, et al: Immunologic purging of marrow assessed by PCR before autologous bone marrow transplantation for B-cell lymphoma [see comments]. N Engl J Med 325:1525-1533, 1991

13. Rowley SD, Zaehlecker M, Braine HG, et al: CFU-GM content of bone marrow graft correlates with time to hematologic reconstitution following autologous bone marrow transplantation with 4-hydroperoxycyclophosphamide-purged bone marrow. Blood 70: 271-275, 1987

14. Gribben JG, Neuberg D, Freedman AS, et al: Detection by polymerase chain reaction of residual cells with the bcl-2 translocs-

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OCT 17 '96 11:24 FROM MED UNIV/MEDICAL ONCOLOGY P.11/11

tion is associated with increased risk of relapse after autologous bone marrow transplantation for B-cell lymphoma. Blood 81:3449-3457, 1993

15. Krause D, Ito T, Fackler M, et al: Characterization of murine CD34, a marker for hematopoietic progenitor and stem cells. Blood 84:691-701, 1994

16. Silvestri F, Banavali S, Baccarani M, et al: The CD34 hemopoietic progenitor cell associated antigen: Biology and clinical applications. Haematologica 77:265-272, 1992

17. Berenson RJ, Bensinger WI, Hill RS, et al: Engraftment after infusion of CD34+ marrow cells in patients with breast cancer or neuroblastoma. Blood 77:1717-1722, 1991

18. Shpall EJ, Jones RB, Bearman SI, et al: Transplantation of autologous CD34+ hematopoietic progenitor cells into breast cancer patients following high-dose chemotherapy. J Clin Oncol 12:28-36, 1994

19. Brugger W, Heimfeld S, Berenson R, et al: Reconstitution of hematopoiesis after high-dose chemotherapy by autologous progenitor cells generated ex vivo. N Engl J Med 333:283-287, 1995

20. Krause DS, Fackler MJ, Civin CI, et al: CD34: Structure, biology, and clinical utility. Blood 87:1-13, 1996

21. Tsay Y-G, Jones R, Calenoff E, et al: A preoperative chymopapain sensitivity test for chemonucleolysis candidates. Spine 9:764-768, 1984

22. Hardwick A, Law P, Mansour V, et al: Development of a large-scale immunomagnetic separation system for harvesting CD34-positive cells from bone marrow, in Gross S, Gee AP, Worthington-White DA (eds): Advances in Bone Marrow Purging and Processing. New York, NY, Wiley-Liss, 1992, pp 583-589

23. Ishizawa L, Hangoc G, Van de Ven C, et al: Immunomagnetic separation of CD34+ cells from human bone marrow, cord blood, and mobilized peripheral blood. J Hematother 2:333-338, 1993

24. Trischmann TM, Schepers KG, Civin CI: Measurement of CD34+ cells in bone marrow by flow cytometry. J Hematother 2:305-313, 1993

25. Civin CI, Strauss LC, Fackler MJ, et al: Positive stem cell selection: Basic science, in Gross S, Gee A, Worthington-White D (eds): Bone Marrow Purging and Processing. New York, NY, Liss, 1990, pp 387-402

26. Strauss L, Trischmann T, Rowley S, et al: Selection of normal human hematopoietic stem cells for bone marrow transplantation using immunomagnetic microspheres and CD34 antibody. Am J Pediatr Oncol 13:217-221, 1991

27. Brodeur GM, Pritchard J, Berthold F, et al: Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J Clin Oncol 11:1466-1477, 1993

28. Brodeur GM, Seeger RC, Schwab M, et al: Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science 224:1121-1124, 1984

29. Brodeur GM, Fong CT: Molecular biology and genetics of human neuroblastoma. Cancer Genet Cytogenet 41:153-174, 1989

30. Canetta R, Franks C, Smaldone L, et al: Clinical status of carboplatin. Oncology 1:61-70, 1987

31. Legha SS, Dimery IW: High-dose cisplatin administration without hypertonic saline: Observation of disabling neurotoxicity. J Clin Oncol 3:1373-1378, 1985

32. Berman M, Feldman S, Alter M, et al: Acute transverse myelitis: Incidence and etiologic considerations. Neurology 31:966-971, 1981

33. Eguro H: Transverse myelitis following chemonucleolysis. J Bone Joint Surg 65:1328-1329, 1983

34. Hirst E, Robertson TI: The syndrome of thymoma and erythroblastopenic anemia. Medicine (Baltimore) 46:225-264, 1967

35. Ladenstein R, Lasset C, Hartmann O, et al: Comparison of auto versus allografting as consolidation of primary treatments in advanced neuroblastoma over one year of age at diagnosis: Report from the European Group for Bone Marrow Transplantation. Bone Marrow Transplant 14:37-46, 1994

36. Moss TJ, Cairo M, Santana VM: Clonogenicity of circulating neuroblastoma cells: Implications regarding peripheral blood stem cell transplantation. Blood 83:3085-3089, 1994

37. Moss TJ, Xu ZJ, Mansour V: Quantitation of tumor cell removal from bone marrow: A preclinical model. J Hematother 1:65-73, 1992

38. Ross AM, Cooper BW, Lazarus HM: Detection and viability of tumor cells in peripheral blood stem cell collections from breast cancer patients using immunocytochemical and clonogenic assay techniques. Blood 82:2605-2610, 1993

39. Champagne MA, Civin CI: CD34+ progenitor/stem cells for transplantation. Hematol Rev 8:15-25, 1994

40. Champagne MA, Amin S, Civin CI: CD34 Positive selection (PS) is effective for cancer purging and purging efficacy (PE) can be calculated from clinically measurable parameters. Blood 80:272a, 1992 (suppl 1, abstr)

41. Matthay KK, Seeger RC, Reynolds CP, et al: Allogeneic versus autologous purged bone marrow transplantation for neuroblastoma: A report from the Childrens Cancer Group. J Clin Oncol 12:2382-2389, 1994

42. Chen AR, Cohen KJ, Hby LL, et al: Heterogeneous mobilization of CD34+ blood stem cells for autologous rescue in children with poor prognosis solid tumors. Blood 86:403a, 1995 (suppl 1, abstr)

43. Smeland EB, Funderud S, Kvalheim G, et al: Effective isolation of human hematopoietic progenitor cells: A new method for detachment of immunomagnetic beads from positively selected CD34+ cells. Int J Cell Cloning 10:108-110, 1992

44. Smeland EB, Funderud S, Kvalheim G, et al: Isolation and characterization of human hematopoietic progenitor cells: An effective method for positive selection of CD34+ cells. Leukemia 6:845-852, 1992